ALS SCIENCE HIGHLIGHT

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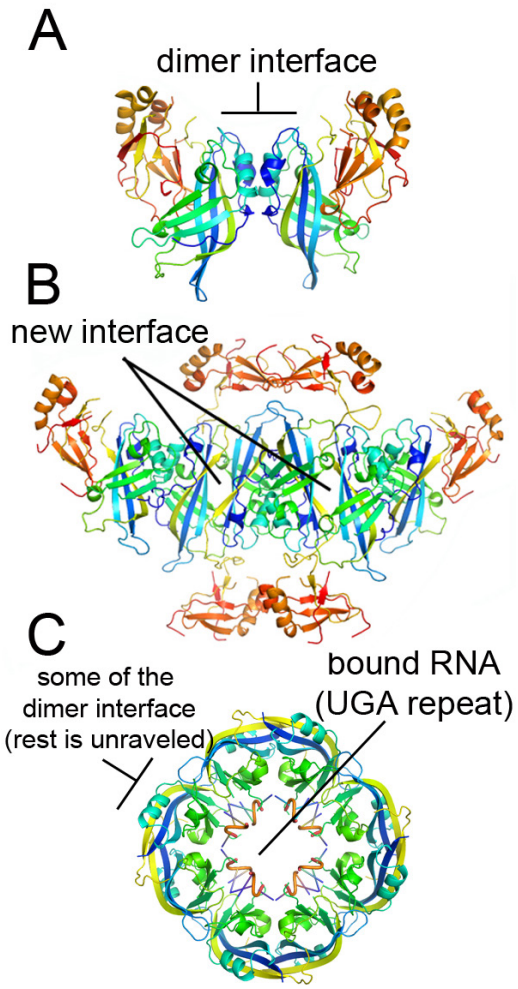
ALS Capabilities Reveal Multiple Functions of Ebola Virus

A central dogma of molecular biology is that a protein's sequence dictates its fold, and the fold dictates its function. Scientists typically expect that a protein has a singular structure (with some conformational variation), and that when an experimental structure is solved, it can used to understand the known biological function(s) of the protein. Recently, researchers used ALS capabilities to demonstrate that a protein of Ebola virus, termed VP40, undergoes dramatic refolding rearrangements to achieve three entirely different structures for three entirely separate functions in the virus life cycle.

Evolution has compelled some viruses, such as Ebola virus, to maintain extremely limited genomes. Indeed, Ebola virus encodes only seven genes in its genome. Yet, the virus achieves many more than seven protein functions in its life cycle. Researchers set out to solve the question of how the Ebola virus does more with less.

They discovered that the answer lies in partial unfolding, rearrangement, and reassembly of the VP40 polypeptide into different forms for different, essential functions as the needs of the virus changed during its life cycle. VP40 appears to initially form an octameric ring to bind RNA



Three structures of VP40, colored blue to red, N to C terminus. (A) A butterfly-shaped dimer of VP40 is essential for trafficking. (B) A hexamer, formed by rearrangement of N- (blue/green) and C- (orange) terminal domains and inversion of central subunits, polymerizes into filaments to build and bud virions. (C) An octameric ring binds RNA to control transcription. No other form of VP40 binds RNA. Some of the residues that form the dimer interface are positioned on the outside of the ring; another 70 are disordered. The C-terminal domains are absent from this crystal structure; EM suggests they extend up and down from the ring.

Publication about this research: Z.A. Bornholdt, T. Noda, D.M. Abelson, P. Halfmann, M.R. Wood, Y. Kawaoka, and E.O. Saphire, "Structural Rearrangement of Ebola Virus VP40 Begets Multiple Functions in the Virus Life Cycle," *Cell* **154**, 763 (2013).

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In Search of a Cure

The Ebola virus outbreak in West Africa has claimed over 110 lives and more than 170 suspected or confirmed cases have been reported. Ebola viruses cause severe hemorrhagic fever with up to 90% lethality and are considered potential biological weapons. While there is no known cure, basic research is providing insight into the action of the virus. Researchers are studying VP40, a multifunctional Ebola virus protein that plays critical roles at different stages of the virus life cycle. To elucidate the VP40 structure and its related function, they crystallized the protein and collected data at three light sources, including the Berkeley Center for Structural Biology (Beamline 5.0.2) at the Advanced Light Source. The researchers analyzed the x-ray data using PHENIX, automated crystallography software for determining macromolecular structure.

Their findings reinforce the desirability of VP40 as a target for anti-Ebola virus drugs. The structure-shifting nature of this protein allows for either targeting by more than one drug or upsetting the balance of the different protein conformations, thereby impeding the viral life cycle. Regardless of the approach taken, this work highlights areas that can be exploited to decrease the virulence of this deadly Ebola virus.

and control transcription, later trafficking to the cellular membrane as a butterfly-shaped dimer, and finally rearranging into zigzagging hexamer to assemble and release progeny virions. Pairing of the x-ray crystallographic structures and protein biochemistry with cellular microscopy and viral replication studies definitively assigned the particular structures to particular, essential functions.

To perform this analysis, researchers built upon seminal VP40 crystal structures published by the Weissenhorn group in 2000 and 2003: a monomer in which N- and C-terminal domains appeared weakly associated, and an RNA-bound octameric ring formed by perturbation of the VP40 protein. At the time, VP40 was only known as the viral "matrix" protein, responsible for assembling the protein shell from which new virions are made. Mutants that prevented formation of the ring structure had no effect on virus budding. Thus, the function of the RNA-binding ring form of VP40 would not become clear for several more years until researchers found that VP40 also controlled viral transcription prior to assembly and release of new viruses. The researchers developed a point mutation that anchors VP40 into its ring structure, and used this mutant to demonstrate that the ring structure, and not a monomer, dimer, or the rearranged hexamer, is responsible for that transcriptional control.

First, the demonstration that VP40 must make and potentially interconvert between multiple distinct structures provides multiple opportunities for antiviral drug design. Drugs that inhibit the ring, the dimer, or the hexameric filament structure, or perhaps the flexibility required to interconvert among the three, would equally disable the virus. Additionally, the rigorous experimental proof that a single, unmodified, wild-type polypeptide doesn't have one characteristic structure, but instead, has multiple, distinct structures — each essential to the organism that encoded it — expands our understanding of capabilities of proteins in general, as well as the layers by which information is encoded in the genome. Such "transformer" proteins as VP40 may be more likely to be found in viruses, because they often make do on little genetic material, but they may be found in cellular organisms as well. Indeed, viral proteins are assembled using cellular amino acids, on cellular machinery, using polypeptide chemistry identical to that of cellular proteins. This research brings up the question of whether there are other transformers or morpheeins that exist in biology and may even be linked to human disease.